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PITFALL TRAPPING IN POPULATION GENETICS STUDIES: FINDING THE RIGHT "SOLUTION"

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ABSTRACT. It is imperative to obtain a representative sample of each population for population genetics studies. Furthermore, it must still be possible to isolate DNA from these organisms. We adapted the pitfall technique for that purpose after encountering severe problems collecting sufficiently large numbers of live *Coelotes terrestris* (Wider 1834) (Amaurobiidae) in the field. Although this species is commonly caught in pitfalls, collecting them by hand proved to be much more laborious than expected. Initially, we tested two types of live-traps (one cup and one funnel trap) which had been successfully used to catch carabid beetles. Both types did not yield enough captures of *C. terrestris* to get a representative sample of the studied populations. Therefore, we tested three different killing/preservative solutions (70 % ethanol, acetic acid + TE buffer and 4% formaldehyde) for possible use in pitfall traps. Ethanol was the best preservative solution based on the amount of DNA that could be isolated after treatment and on the ability to generate the same RAPD profile as a reference DNA sample preserved at −20 °C. To test ethanol as a preservative solution in the field, we varied its concentration and used it in combination with traps with or without funnels. We conclude that it is best to use a funnel trap with 96% ethanol. We further recommend that for every new species to be sampled in this way an explorative investigation should be carried out determining where, when, and how many traps should be placed (this reduces the expense of the method). Furthermore, the effects of different preservative solutions on the DNA of an organism of interest should be tested. The resolution of the molecular analysis will determine if the DNA should be of high-molecular-weight or if some degree of denaturation is allowed.

Keywords: Pitfall trapping, DNA preservation, *Coelotes terrestris*, population genetics, Araneae

The possibility of using spiders as bio-indicators for nature conservation measures such as assessing effects of habitat fragmentation was proposed in Maelfait (1996), Maelfait & Baert (1997) and Maelfait & Hendrickx (1998). We are investigating possible population genetic effects from forest fragmentation in Flanders (Belgium) on the spider *Coelotes terrestris* (Wider 1834) (Amaurobiidae). This model organism was chosen because it is strongly bound (stenotopic) to forest habitats based on its way of prey capture and web building (Tretzel 1961). Furthermore, it is one of the most abundant spider species in forests on loamy or sandy loam soils (De Bakker et al. 2000).

It is imperative to obtain a representative sample (e.g., 30 individuals) of each population for population genetics studies. Furthermore, it must still be possible to isolate DNA from these organisms. We encountered severe problems during sampling campaigns collecting sufficiently large numbers of live spiders in the field. Although this species is among the most commonly caught spiders in pitfalls, collecting them by hand proved to be much more laborious than expected. Because the spider is night active, it mostly remains hidden during the day in places not easily accessible, making them extremely difficult to find and collect.

It is indeed possible to catch large numbers of *C. terrestris* in a short period of time by means of pitfall traps filled with formaldehyde (Segers & Maelfait 1990). Most individuals are caught from August through October and

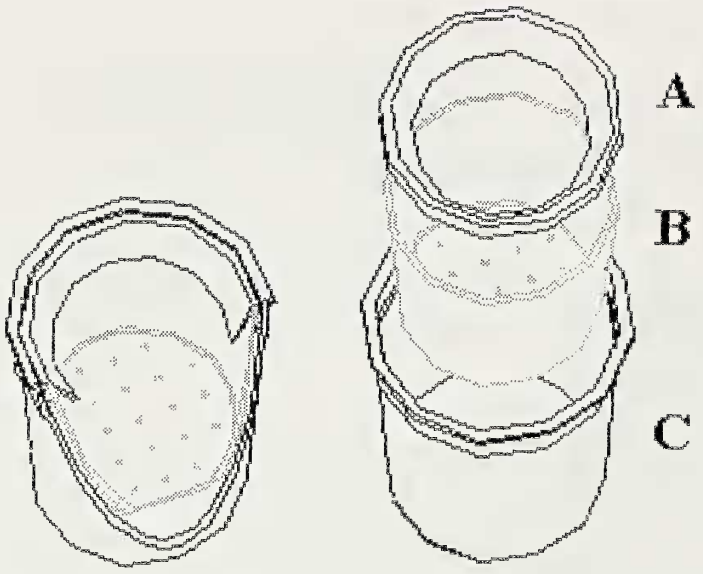

Figure 1.—Pitfall trap Type I (A: plastic rim, B: small receptacle and C: large cylinder) to collect *Coelotes terrestris*.

are adult males actively searching for females. Pitfall traps are commonly used to investigate abundance, activity, and distribution of epigeal arthropods. The method is very attractive because it is not labor-intensive to catch a large amount of organisms and it is inexpensive (Maelfait & Baert 1975; Topping & Sunderland 1992). The possibility to use it for population genetics studies, however, was to our knowledge, never tested.

The challenge of this research was to find (1) a suitable trap type that makes it possible to collect a representative sample of the populations and (2) a trapping solution that does not affect DNA quality. This would strongly reduce the need to use hand catches or even make them superfluous.

METHODS

Description of the traps.—We tested two types of traps. The cup trap (Type I, Fig. 1) was provided by Dr. H.-J. Vermeulen (Netherlands), who used it to collect ground-active carabid beetles. It consists of one large plastic cylinder (diameter 11 cm, 15 cm deep) that, by means of a black plastic rim, holds a small plastic receptacle to collect the organisms (10 cm diameter, 9.5 cm depth, with small drainage holes for the rain water). The trap was used as a live-trap and was one-fifth filled with dead leaves to create hiding places. We sampled 10 forests with 9 traps that were 3 m apart and in a square of 6 by 6 m. The traps were used in August and September 1999 and were emptied every fortnight.

The Type II trap consists of a plastic bottle

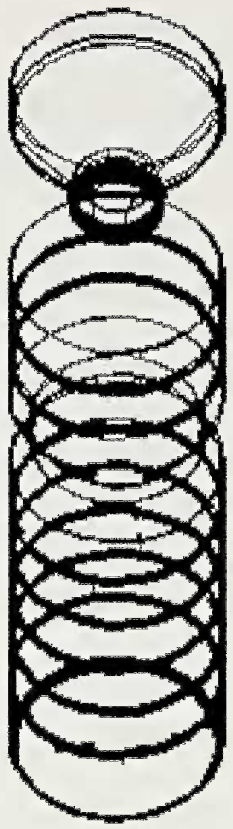

Figure 2.—Pitfall trap Type II & III: plastic water bottle with cut-off top to collect *Coelotes terrestris*.

with a cut-off top (diameter 7.5 cm, depth 20 cm, Fig. 2) and was one-third filled with ethanol. In the Type III trap we used the cut-off top as a funnel in the plastic bottle. The funnel-neck had a diameter of 2.5 cm. The funnel trap was used as a live-trap and half-filled with dead leaves to create hiding places for the captured organisms (Type IIIa). It was also used in combination with ethanol (Type IIIb).

From August to October 2000, 16 sites in 13 forests were sampled with 3 traps that were 3 m apart. The different trap types and solutions were tested consecutively during the sampling campaign (Table 1). The traps were emptied weekly.

All traps were placed in the interior of the

Table 1.—Scheme of the *Coelotes terrestris* sampling campaign. Each week, sixteen sites were sampled with 3 traps per site.

Week	Date
1	Type IIIa
2	Type IIIa
3	Type IIIa
4	Type IIIb-96% ethanol
5	Type IIIb-96% ethanol
6	Type II-96% ethanol
7	Type II-75% ethanol
8	Type II-85% ethanol

forest, under the trees. They were sunk in the ground with their rim level to the soil surface. About 3 cm above each trap was a 15x15 cm PVC roof to guard it from excessive rain, leaves or other debris. There were also plastic strips (height 3 cm, length 25 cm) on two opposing sides of the trap to guide the spiders into the trap.

All investigated forests were deciduous and mainly beech (*Fagus sylvatica*) forests, occurring on sandy loam or loamy soils and with a litter layer of the *moder*-type. They are old forests occurring on the maps of De Ferraris (1772–1779), the oldest topographical maps of Flanders. The choice of sampling sites in the forest was based on an earlier inventory study by De Bakker et al. (2000).

Testing preservation solutions.—We used eight individuals of *C. terrestris* to test three solutions: 70% ethanol (diluted from 96% ethanol), a 1:1 mixture of acetic acid:TE buffer (Tris + EDTA) and 4% formaldehyde. For each individual, one pair of legs was kept in 1ml of each of the three solutions for 2 months at room temperature. The fourth pair of legs was stored at −20 °C as a control sample.

Formaldehyde and acetic acid + TE (derived from Carnoy's solution: 60% ethanol, 30% chloroform, 10% acetic acid, pH 2.6) were tested as less expensive alternatives to ethanol. We especially wanted to test the short-term storage effect of these solutions on the DNA of *C. terrestris*.

Genomic DNA was extracted with the PureGene DNA isolation kit (Type D-5000A, Gentra Systems, Inc., Biozym, Landgraaf, The Netherlands), following the manufacturer's instructions. The isolated DNA was quantified with a spectrophotometer and brought to a final concentration of 5 ng/μl for further analysis. The RAPD technique (Random Polymorphic DNA, Welsh & McClelland 1990; Williams et al. 1990) was conducted as described in Gurdebeke et al. (2000) with primer OPA-01 (Operon Technologies Inc., Alameda, California, USA) to check the quality of the isolated DNA.

Testing ethanol in the field.—To test ethanol as a preservative solution in the field, we varied its concentration (96%, 85% and 75%) and used it in combination with traps without (Type II) or with a funnel (Type IIIb). All dilutions of ethanol were made from a non-denatured 96% stock solution. The traps were emptied every week and the organisms were kept in 70% ethanol at −20 °C prior to DNA isolation. The DNA was isolated within one month after catching the spiders and the quality was checked during a 0.8% agarose gel electrophoresis.

For every sampling site, the proportion of *C. terrestris* caught with Type II, Type IIIa and Type IIIb traps was calculated. By using proportions, we can correct for differences in abundance between different sampling sites. To correct for possible phenology-related differences in the number of caught individuals, we only used the data of week 3 (Type IIIa), 5 (Type IIIb) and 6 (Type II). A non-parametric Friedman ANOVA tested for significant differences in capturing rates between the trap types (StatSoft, Inc. 2000).

Voucher specimens were deposited in the Royal Belgian Institute of Natural Sciences in Brussels, Belgium (identification number IG 29.487).

RESULTS

Live-traps.—Although very suitable for trapping carabid beetles (Vermeulen 1994), the cup traps filled with leaves (Type I) yielded fewer than 10 *C. terrestris* in all traps in the 10 forests where the traps were used. The funnel trap filled with leaves (Type IIIa) gave variable results. In only one of the sixteen sampling sites, a representative sample of the population (being at least 30 individuals) was caught in 3 weeks, but no individuals were captured in 13 of the sampling sites. Despite the leaves, occasional killing still occurred when more than one spider was caught in one trap. These results made it necessary to investigate the possibility of using a fluid in the pitfall traps that would preserve the DNA of the captured spiders.

Preservation solutions.—Spectrophotometric quantification of the DNA showed that no DNA could be isolated from the samples stored in acetic acid + TE buffer (Table 2). During DNA isolation, we also noticed that those legs were more brittle than legs stored in other solutions. The samples stored in ethanol and formaldehyde both yielded sufficient DNA, however, the amount of isolated DNA was lower than that isolated from legs that were kept at −20 °C. The quality of the DNA was assessed after subjecting the samples to

Table 2.—Amount of isolated *Coleotes terrestris* DNA after different treatments (* isolation failed).

Individual	Control (−20°C) (ng/μl)	70% ethanol (ng/μl)	4% formaldehyde (ng/μl)	Acetic acid + TE (ng/μl)
1	65.50	90.25	12.00	0
2	76.00	74.25	314.25	0
3	111.50	10.75	21.00	0
4	113.00	—*	34.75	0
5	222.50	82.00	4.25	0
6	187.00	30.00	25.75	0
7	116.00	36.25	3.25	0
8	142.00	99.75	8.00	0
Mean	129.19	60.46	52.91	0
st.d.	53.25	34.33	106.17	0

RAPD analysis. It was impossible to generate a RAPD profile from the samples kept in formaldehyde, while the samples stored in 70% ethanol yielded the same banding profile as the control sample that was kept at −20 °C (Fig. 3).

Testing ethanol in the field.—The performance of the traps was evaluated based on the following criteria: the efficiency of the trap (catching and retaining a sufficient number of organisms) and the ability to preserve DNA so that no degradation takes place. We include the live-trap of Type IIIa again in the interpretation of these results. Friedman ANOVA proved the capture rates of the three trap types being significantly different ($P < 0.0001$; Fig. 4).

Live-traps with funnels (Type IIIa) caught on average 5.92 ± 3.29% of the individuals. They were the least efficient with regard to the number of captured organisms. In contrast, DNA of the few animals that were caught alive in these traps and then stored at−20 °C was well preserved and was always of high molecular weight. Funnel traps filled with ethanol (Type IIIb, 96% ethanol) caught more individuals (32.66 ± 6.19%) and DNA quality of the spiders was good and showed no degradation (Fig. 5). The most individuals (61.42 ± 6.67%) were caught with ethanol traps without funnels (Type II, 96% ethanol). However, these traps did not preserve the DNA well enough to yield non-degraded high-molecular-weight DNA.

The DNA of organisms caught with traps filled with 85% and 75% ethanol was also not well-preserved and yielded no or totally degraded DNA.

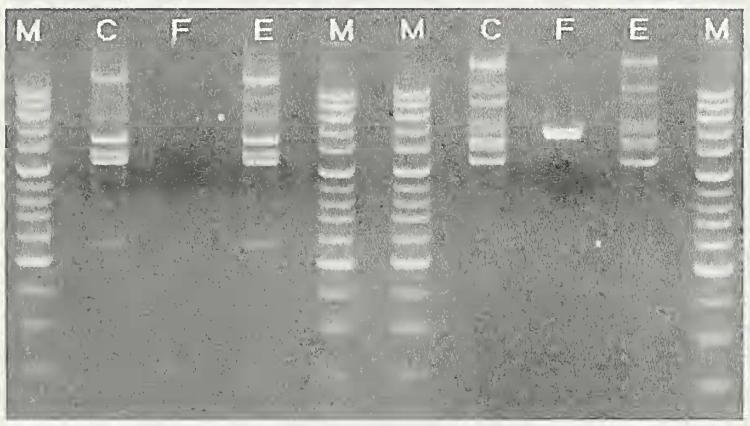

Figure 3.—Banding profiles of 2 *Coelotes terrestris* individuals, generated by RAPD with primer OPA-01 after the different treatments (M: molecular marker, C: control−20 °C, F: formaldehyde, E: 70 % ethanol). It was impossible to generate a RAPD profile from the samples kept in formaldehyde, while the samples stored in 70% ethanol yielded the same banding profile as the control sample that was kept at −20 °C.

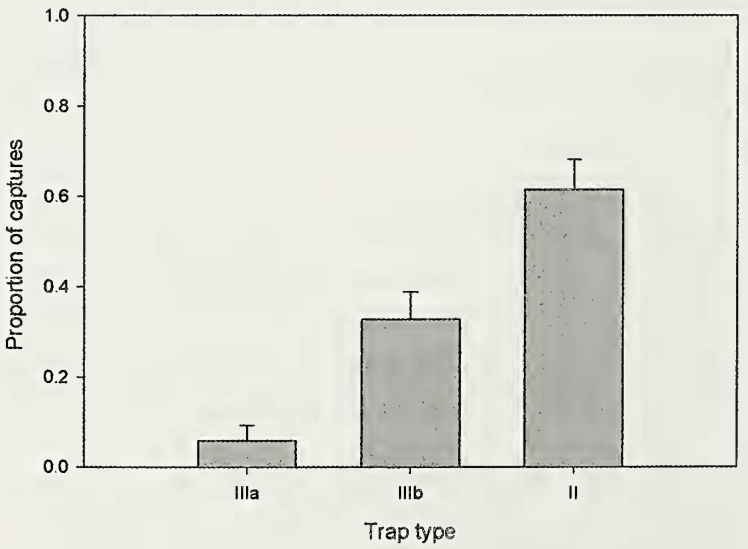

Figure 4.—Proportion of *Coelotes terrestris* captures in traps of Type II, IIIa and IIIb. Capture results are significantly different between the three trap types (Friedman ANOVA, $P < 0.0001$).

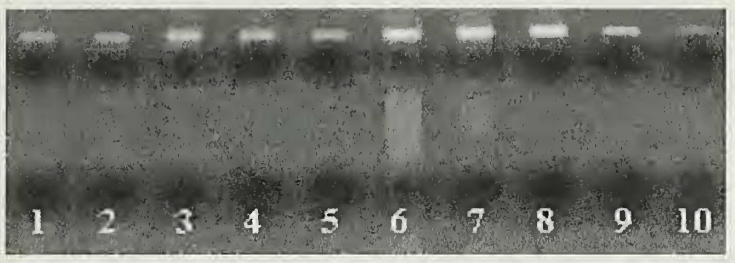

Figure 5.—Quality of isolated *Coelotes terrestris* DNA caught in pitfall traps with funnel and 96% ethanol. Each track represents total genomic DNA of high molecular weight from one individual.

DISCUSSION

Live-traps.—We first tried dry traps because these capture animals alive to be subsequently frozen to preserve their DNA for further molecular-genetic analysis. None of the two live-traps yielded enough organisms to get a representative sample of the studied populations. This was probably due to a high escape rate of the spider from the trap. Linyphiid spiders may escape from (even fluid-filled) pitfall traps (Topping 1993), but this remains an open question for *C. terrestris*. Adding a fluid to the trap prevents spiders from escaping, making the trap more efficient (Curtis 1980; Topping & Luff 1995). This was also observed in this study.

The cup traps caught fewer animals than funnel traps. Obrist & Duelli (1996) recommended funnel traps to collect epigeal arthropods, because they are more efficient than cup traps. The funnel was said to lower the incidence of escape. The small depth might also cause the disappointing results of our cup traps. Adis (1979) recommends traps at least 12 cm deep.

Another factor that might influence the escape from the dry traps is that the traps are made of plastic. Re-using plastic traps roughens the trap surface and may affect the capture (and maybe also the retaining) efficiency of the trap (Topping & Luff 1995). According to Luff (1975) and Waage (1985), escape from empty plastic traps is higher than from empty glass traps for beetles. This is presumably also the case for ground-active spiders.

Preservation solutions.—Degradation of DNA occurs when endo- and exonuclease activity cleaves the DNA strand and breaks it up in small fragments which are no longer suitable for further molecular analysis (Linn 1981). Ester linkages with phosphate molecules and carbon–nitrogen linkages are especially susceptible to modifications in DNA (Cann et al. 1993). Storing samples at −20 °C or −80 °C can inhibit the activity of the nucleases. This method is very cheap and fast and therefore the most widely used. However, some research has also been done to preserve DNA in a chemical solution (see below).

In this study, ethanol was found to be the best chemical preservative based on the amount of DNA isolated after treatment and the ability to generate the same RAPD profile as a reference DNA sample preserved at −20°C. Animal tissue DNA preserved in ethanol may give successful PCR amplification (Dessauer et al. 1996; Dick et al. 1993), but substantial degradation for spider DNA was found after storage for three weeks in 70% ethanol and ethylene glycol at room temperature (A'Hara et al. 1998). This degradation was also reported by Seutin et al. (1991), Holzmann & Pawlowski (1996) and Zhang & Hewitt (1998).

Acetic acid with TE buffer, the solution derived from Carnoy's fixative (60% ethanol, 30% chloroform, 10% acetic acid, pH 2.6) gave the worst results of the three tested solutions. It was impossible to isolate DNA from the legs stored in this fixative. Disappointing results with Carnoy's fixative were also reported by Post et al. (1993) and Koch et al. (1998). It is however possible to use Carnoy's fixative when only specific target sequences need to be amplified from fixed tissues (Honma et al. 1993; Li et al. 1995).

The negative effect of formaldehyde on DNA found by others (Dessauer et al. 1996; Jackson et al. 1991; Holzmann & Pawlowski 1996) was also observed in this study. This can explain why no or only a strongly reduced RAPD banding profile was found, since the RAPD technique needs non-degraded, high-molecular-weight DNA. In contrast, buffered formalin gave good results for targeting specific sequences (Honma et al. 1993) or for the extraction of high-molecular-weight DNA for Southern blot analysis (Koshiba et al. 1993).

A variety of preservation solutions have been tested, sometimes with contradictory results. Seutin et al. (1991) recommended saline solutions; Fukatsu (1999) had good results with 2-propanol (but Post et al. (1993) did not), ethyl acetate (but Reiss et al. (1995) did not) diethyl ether and acetone. Methanol (Post et al. 1993; Fukatsu 1999) and chloroform

(Fukatsu 1999) showed poor DNA preservation. Thus, contradictory findings exist concerning which specific solution is good for DNA preservation. One should test the effect of a particular DNA fixative on the organism of interest before using it as a storage medium. The resolution of the molecular analysis will determine if the DNA should be of high-molecular-weight or if some degree of denaturation is allowed.

Testing ethanol in the field.—One has to consider two aspects when using ethanol as a preservative solution in the field: the high evaporation rate of this solution and the expense of using (nearly) absolute ethanol. An ethanol concentration of 70% proved to have enough preservative capacities in the lab. However, when 75% or even 85%, was used in the field, the DNA of the spiders showed massive degradation. It seems that a certain threshold concentration is necessary to prevent the DNA from losing its original structure. The concentration of 70% or 85% ethanol probably became diluted through evaporation in the field, causing it to lose its preservative properties. Using both concentrations in a trap with a funnel to minimize evaporation gave no better results.

When using absolute ethanol in the field (96%), we did see an effect of using a funnel trap to prevent evaporation. Although the DNA of spiders caught in cup traps filled with 96% ethanol was not intact, the DNA of those captured in funnel traps was. Therefore, we conclude that it is best to use a funnel trap with 96% ethanol.

It is recommended that for every new species to be sampled in this way an explorative investigation should be carried out determining where, when, and how many traps should be placed (this reduces the expense of the method). Furthermore, the effects of different preservative solutions on the DNA of an organism of interest should be tested.

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